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A METHOD FOR DETERMINING THE GERMICIDAL VALUE AND PENETRATING POWER OF LIQUID DISINFECTANTS.*

ARTHUR I. KENDALL AND MARTIN R. EDWARDS.

(From the Department of Preventive Medicine and Hygiene, Harvard Medical School.)

The ever-increasing use of disinfectants, following, logically, the interest manifested by communities and individuals in the control and suppression of communicable diseases, has been accompanied by a remarkable development in the manufacture of these substances.

Coincident with this increased use and production of disinfecting agents there has arisen a natural demand on the part of the public for information regarding the relative merits of these various germicides, and various attempts have been made in the past to formulate methods which should furnish information relating to their germicidal powers. Prior to the publication of the Rideal-Walker method no satisfactory test had been evolved which would give concordant results in the hands of different workers.

The lack of success which has attended these previous investigations appears to have been due to the non-appreciation of the influence of certain factors and details which should be integral parts of such procedures, and which when properly controlled should lead to constancy in results. Of the various methods which have been proposed and employed, that known as the Rideal-Walker coefficient or, as it was first termed, the carbolic coefficient, takes these factors most fully into consideration, and the constancy of results attainable with this method depends in no small degree on the recognition and control of these variable elements.

Among the most important factors considered in the method are: (1) the time of exposure of the test organism to the germicide; (2) the age of the culture employed; (3) the choice and reaction of the medium in which the test organisms are grown; (4) the temperature of incubation; (5) the temperature of medication; (6) the use of a

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* Received for publication January 5, 1911.

24-hour-old typhoid culture in standard broth; (7) the proportion of culture to disinfectant.

The Rideal-Walker coefficient is a method for determining by direct comparison the relative germicidal values of different disinfectants upon naked germs in the absence of more than minimal amounts of organic matter. Carbolic acid is the standard to which the disinfecting power of the various germicides is referred. This method is of value in determining the bactericidal power of only one class of germicides—those employed in the disinfection of surfaces and substances contaminated by pathogenic organisms; it is not applicable for testing the efficiency of gaseous disinfectants. The procedure, therefore, is definitely limited in application to that group of germicides which are employed as solutions or emulsions for the disinfection of contaminated liquids or solids. Among the more commonly met with instances in which these liquid or emulsified disinfectants are employed are for alvine discharges, fomites, urinary discharges, for privies and such surfaces as floors, walls of stables, and so on.

A brief consideration of the conditions bringing about this type of contamination will reveal the fact that purely superficial infection will be an exception rather than the rule, and consequently the ability to penetrate is one of the most important properties that an efficient germicide of this class can possess.

The method here outlined takes into consideration the salient features of the Rideal-Walker method, and in addition furnishes a means for estimating the relative powers of penetration of these liquid disinfectants. The method consists essentially in the exposure of test organisms in infected cylinders of agar of definite diameter, to the germicide for definite periods of time. At stated intervals the agar cylinders are removed from the disinfecting solutions and washed with sterile water. A plug of agar about 3 mm. in diameter is then removed from the cylinder, or cylinders, by means of quill tubing. This plug of agar is taken from the center of the agar cylinder, along its long axis. It is placed in sterile broth and incubated at the body temperature for several days, during which time observations are made to determine the presence or absence of growth. By comparing the results obtained with a given disinfectant and those obtained under the same conditions with carbolic acid as a standard, a ratio may be established which expresses in terms of carbolic acid the combined germicidal and penetrating power of the bactericidal substance in question.

It is essential to discuss briefly the various steps which led to the method which has been outlined above.

The first attempt to measure the penetrating power of disinfectants consisted in pouring the germicide upon the surface of a solidified agar shake culture maintained in an upright position. The disinfectant was allowed to remain on the top of the agar for a definite time, then poured off, and the tube inverted to permit the excess to drain away. This procedure gave no definite results.

The next step was a modification of the foregoing in which dextrose agar and a gas-forming bacillus were used. Differences in penetrating power could be discerned, but accurate comparisons were impossible. This appeared to be due, partly at least, to the fact that but one surface of the agar was exposed to the solution. This method did, however, show very definitely the advantage of gas production as an indicator in those cases in which the germicide failed to penetrate completely.

In order to take advantage of this gas production and at the same time to allow penetration from all directions, agar containing dextrose, and infected with colon bacilli, was poured into long cylindrical glass tubes (sterile of course) of definite diameter, and allowed to harden thoroughly. The agar was then forced out and cut into lengths slightly greater than the diameter. These fell directly into the germicidal solutions, the time at which they entered being noted. At the end of stated intervals some of these cylinders were removed, washed, and placed in sterile water in which they were incubated at body temperature. They were examined daily for several days to observe growth.

Any development of the colon bacilli was demonstrated by the production of gas as shown by the splitting of the agar. The method proved to be satisfactory for showing the combined inhibitory and penetrating power of these disinfectants, but it soon became apparent that a distinction must be drawn between the power of inhibition and the actual killing of the test organisms.

It is obvious that the penetrating germicide will reach those organisms in the centers of the cylinders last; hence, if a core be removed from the agar cylinder along its long axis in the center, and this core or plug be placed in dextrose broth, a production of gas will indicate that the germs in the center of the agar cylinder were not killed by the germicide, and consequently that the germicide was not efficacious. It is essential that there shall be a considerable volume of broth in proportion to the size of the core of agar, to dilute the disinfectant which has penetrated the ends of the core when the agar was exposed to the germicide.

The last modification adopted consisted of using plain agar, and *B. coli* as a test organism. The plugs or cores were placed in dextrose broth for incubation, and the development of gas showed that the test organisms were not killed.

The cultures used in the experiments were made from 24-hour-old growths in standard broth: 10 c.c. of this broth culture were added to each liter of agar, and the two were thoroughly mixed. This infected agar was formed into a long cylinder by pouring it into long sterile tubes, and allowing it to harden in the vertical position. The ends of the tube were closed with sterile rubber stoppers.

The diameter of the tubes in which the infected agar is hardened is a point of great importance, since the germicides must penetrate a distance equal to one-half the diameter of the cylinders of agar to kill the organisms in the center. The diameter finally selected was 15 mm.; tubes ranging from 8 to 30 mm., however, were tried before deciding upon the 15 mm. tube. This size of tube was found to be particularly satisfactory in those experiments in which it was desired to bring out both the inhibitory and the killing times of the germicides. The zone between the inhibitory and the killing power is less marked in the 8 mm. tubes, and is greatly drawn out in the larger sizes. With the larger agar tubes, again, it was found that a much longer exposure to the germicides was necessary to actually kill the test organisms.

After the tubes of infected agar were hardened, it was possible to push out the agar in the form of a long cylinder, and as the agar cylinder emerged from the glass tube, it was cut into lengths a little longer than the diameter of the agar cylinder and these short lengths in turn were dropped, as they were cut, into the disinfectant solutions. A sufficient number of such cylinders were dropped into each solution to provide a separate one for each time interval in which it was desired to make a test. At the end of each time interval, a cylinder of agar was removed, washed with sterile water (or water free from gas-forming organisms), and a core about 3 mm. in diameter was removed from the center along the long axis, with a piece of quill tubing, and placed at once in dextrose broth. The dextrose tubes (fermentation tubes) were then incubated at body temperature, and gas formation was looked for upon several successive days.

The time intervals should be a matter of hours rather than of minutes, inasmuch as shorter intervals than an hour seldom showed more than an inhibitory action.

Several technical details need further comment: The agar cylinders that have been exposed to the germicidal solutions are most conveniently handled in holders made of wire gauze, bent up at one end in such a manner as to form a cradle of appropriate size. The pieces of gauze from which these holders are made should be at least 6 inches long and 1 inch wide, to allow for a handle on each. Several of these holders are sterilized in boiling water and kept for use.

The cores of agar removed from the cylinders by means of the quill tubing are readily forced into the fermentation tubes prior to incubation simply by blowing on the free end. By so doing the cores will readily slip from the tubing into the fermentation tubes.

The advantage of using *B. coli* for a test organism is a twofold one: the organism is very readily obtained, and the gas formation in dextrose or lactose broth furnishes a ready method of distinguishing it from accidental contaminating organisms, should such occur. Almost any organism, however, can be used as a test organism to suit special cases.

The temperature at which the exposure to the disinfectants is made should be 20° C.—a temperature readily gained and maintained in the ordinary laboratory.

One decided advantage of this method, aside from those mentioned, is the fact that it is possible to add almost any organic substance (or even feces) to the agar before it is allowed to harden. This addition may be made at a temperature below the coagulating point of most albuminous bodies.

It should be stated that there are two possible objections to the procedure: In the first place it may be argued that agar is a substance not met with in nature, and that the results obtained might conceivably not be applicable in practice. The other objection is that an error due to unavoidable densities of various samples of agar might render the results inconstant.

To the first objection it can be stated that it would be very difficult to find any one substance that would fulfil all requirements; the fact that various organic materials can be added as desired makes the method one of general if not of universal application. The procedure, furthermore, is designed to test the relative penetrating and germicidal values of disinfectants, and for this purpose it appears to be of real value.

The density of the agar can be determined, if necessary, with a modification of the well known Vicat needle, as suggested by Whipple.

In spite of the rather lengthy description necessary to elucidate the workings of this method, it is in reality simpler and more readily carried out than the Rideal-Walker coefficient.

In its present form, this procedure is not regarded by the writers as a perfect method; doubtless modifications and additions will be introduced which will add much to its completeness. On the other hand, the inference must not be drawn that it is an untried method. The results obtained have been reasonably constant, and the trials sufficiently numerous to justify its presentation.

In the appended table, several features are worthy of note.

Formaldehyde, even in relatively weak solutions, appears to be the most efficient of the germicides tested. Carbolic acid, in corresponding dilutions, is less efficient.

The chloride of lime tested here was bought in the open market, and was found to be deficient in chlorine.

The emulsions, of which hyco and sulphonaphthol are examples, are especially noteworthy. These germicides are without doubt efficient when acting upon naked germs, and consequently give high Rideal-Walker coefficients, but are relatively useless when their penetrability is considered. The most logical explanation of this fact is that the very nature of their physical state—emulsions—is incompatible with penetrating power.

In conclusion, the writers wish to express their heartiest appreciation to Dr. Rosenau for his many helpful suggestions. It is fair to state that many of the most essential features of the procedure outlined above are the result of his friendly criticisms.

The procedure finally adopted was as follows:

1. Prepare a 24-hour culture of *B. coli* in plain broth of standard composition and reaction.
2. Add 10 c.c. of this standard culture to one liter of agar [1.5 per cent agar] and mix thoroughly.
3. Pour the infected agar into sterile tubes of convenient length [1 meter] and of exactly 1.5 cm. diameter and allow to harden, after closing the ends with sterile rubber stoppers.
4. Allow to harden at 20° C.
5. Prepare dilutions of the desired disinfectants and a standard 5 per cent carbolic acid solution. The latter is the standard to which the other disinfectants are referred.
6. Place the disinfectants so prepared in sterile beakers, allowing 50 c.c. for each agar cylinder (described below).
7. Remove stoppers from the infected agar tubes, and permit the contents to run out slowly as a long cylinder; with a sterile knife cut off portions by transverse cuts of 2 cm. length, and allow these smaller cylinders to fall directly into the disinfectant solutions, one cylinder for each time interval selected.
8. Note the temperature of the solutions. They should be kept at exactly 20° C. during the experiment.
9. Note the time at which the cylinders were dropped into the disinfectant solutions.
10. At the end of stated intervals (usually at hourly intervals for preliminary tests) remove one cylinder from each solution with one of the sterile holders mentioned above, wash it thoroughly with sterile water, and remove a core from the center with a sterile piece of quill tubing (3 mm. bore).
11. Place these cores in lactose fermentation tubes, after they are properly labeled, and incubate at 37° C. for several days, making daily observations.
12. Compare the killing times of the various solutions tested with that obtained for carbolic acid, and determine the carbolic coefficient of germicidal and penetrating powers combined.

The appended table shows the results obtained with several well known germicides. They illustrate the general method of stating results. The results presented here represent preliminary tests;

final tests are made by studying the killing power in shorter time intervals, utilizing the data obtained in the first examinations.

TABLE SHOWING COMBINED GERMICIDAL AND PENETRATING POWERS OF SOME COMMON DISINFECTANTS.

Agar 1.5 per cent. 72-hour incubation at body temperature. Temperature of exposure, 20°.

DISINFECTANTS	DILUTION	TIME OF EXPOSURE		
		1 hour	3 hours	5 hours
Carbolic acid	5 per cent	+	+	−
Carbolic acid	1 “ “	+	+	+
Formalin	4 “ “	−	−	−
Formalin	1 “ “	+	−	−
Formalin	0.25 “ “	+	+	−
$HgCL_2$	0.1 “ “	+	+	+
$HgCL_2$	1 “ “	+	−	−
Chloride of Lime	10 “ “	+	+	−
Chloride of Lime	4 “ “	+	+	+
Hyco	2 “ “	+	+	+
Cresol	1 “ “	+	+	+
Sulphonaphthol	2 “ “	+	+	+

+ = growth.
− = no growth.